EXHIBIT 35

[54] Mapping Viral mRNAs by Sandwich Hybridization

By ASHLEY R. DUNN and JOSEPH SAMBROOK

Messenger RNAs are most commonly assigned to specific genomic locations by demonstrating that they are complementary to a restriction fragment of DNA whose position within the genome is known. Hybridization to two adjacent fragments is usually taken to mean that a particular mRNA contains sequences which are contiguous within the genome and which span the restriction endonuclease cleavage site. However, this conclusion is fragile because the possibility exists that the mRNA preparation contains two or more species which happen to be complementary to adjacent genomic fragments. In eukaryotic systems, the interpretation is clouded further because mature mRNAs generally are spliced and consist of sequences derived from noncontiguous genomic regions. The technique of sandwich hybridization[1] eliminates some of these problems, and it provides a biochemical method to determine whether sequences from different regions of a genome are covalently joined to one another in mRNA.

In theory, the technique is of general application and can be used to analyze the transcription products of any segment of DNA whose restriction maps are known. However, we have found the technique particularly valuable for mapping a unique class of viral mRNAs which are generated during the course of lytic infection with a number of Ad2–SV40 hybrid viruses and consist of covalently linked adenoviral and SV40 sequences. The genomic structure and transcription map of one such adeno–SV40 hybrid virus, Ad2$^+$ND1, is shown in Fig. 1.[2,3] When such RNAs are hybridized to defined fragments of SV40 or adenoviral DNA immobilized on nitrocellulose filters, the 3′ or 5′ end of the RNA protrudes as a single-stranded tail. The sequences contained within the tail can be determined by a second round of hybridization using ^{32}P-labeled fragments of viral DNA as probes. This is illustrated schematically in Fig. 2.

Experimental Procedures

Isolation of RNA.[4] Cytoplasmic extracts are prepared from monolayers of infected cells or from infected suspension cultures. Washed cell pellets

[1] A. R. Dunn and J. A. Hassell, *Cell* **12**, 23–36 (1977).
[2] A. M. Lewis, M. J. Levin, W. H. Weise, C. S. Crumpacker, and P. H. Henry, *Proc. Natl. Acad. Sci. U.S.A.* **65**, 1128–1135 (1969).
[3] E. Southern, *J. Mol. Biol.* **98**, 503–518 (1975).
[4] E. A. Craig and H. J. Raskas, *J. Virol.* **14**, 26–32 (1978).

ISBN 0-12-181965-5

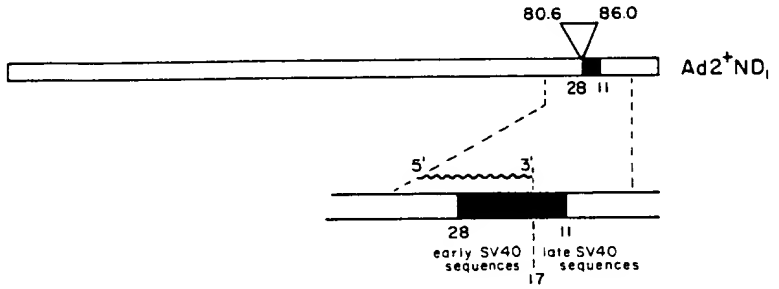

FIG. 1. The genome structure of Ad2⁺ND1. Ad2⁺ND1, originally isolated by Lewis *et al.*,[2] contains a 0.94 kilobase insertion of SV40 DNA (SV40 map coordinates 28–11 shown in black) which replaces 1.9 kilobases of the Ad2 genome located between position 80.6 and 86 on the conventional physical map of adenovirus type 2. In the expanded part of this figure the hybrid transcript is shown with its 5′ end in adenovirus 2 and its 3′ end in SV40 sequences.

are resuspended in 5 volumes of isotonic buffer (0.15 *M* NaCl, 10 m*M* Tris-HCl, pH 7.5, 0.001 *M* EDTA) and allowed to swell for 10 min at 0°. After the addition of Nonidet P40 (NP40) to a final concentration of 0.5% and mixing by vigorous pipetting, nuclei are pelleted by centrifugation at 1000 rpm for 10 min at 0°. The cytoplasmic extract is cleared by centrifugation at 10,000 rpm for 20 min at 0°, adjusted to 0.2% SDS and 0.001 *M* EDTA, and extracted twice with phenol (saturated with 0.5 *M* Tris-HCl, pH 7.5, 0.001 *M* EDTA, 0.15 *M* NaCl) and once with chloroform. RNA is stored at −20° as an ethanol precipitate.

Agarose Gel Electrophoresis. Fragments of viral DNA generated by cleavage with restriction endonucleases are separated by electrophoresis through slab gels[5] (17 × 15 × 0.4 cm) cast with 1–1.2% agarose. After electrophoresis, the contents of the gel are denatured *in situ* (see the following) and transferred to nitrocellulose filters using the Southern blotting technique.[3]

Separation of Viral DNA Strands. For some purposes, hybridization to separated strands of DNA is required. In this case, fragments of viral DNA generated by cleavage with restriction endonucleases are separated by preparative electrophoresis through 1% agarose gels.[6,7] Strips of agarose containing specific fragments are cut from the gel and the DNA denatured *in situ* by immersion in 0.3 *M* NaOH for 30 min at room temperature. After rinsing in several changes of distilled water, the agarose strips are loaded on preformed gels cast with 1.4% agarose such that the length of one side of the agarose strips is in direct contact with the surface of the gel. A little

[5] F. W. Studier, *J. Mol. Biol.* **79**, 237–248 (1973).
[6] G. S. Hayward, *Virology* **49**, 342–344 (1972).
[7] P. A. Sharp, P. H. Gallimore, and S. J. Flint, *Cold Spring Harbor Symp. Quant. Biol.* **39** 457–474 (1974).

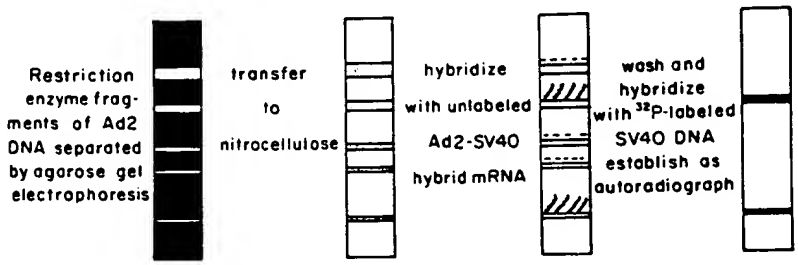

FIG. 2. Schematic representation of sandwich hybridization. Restriction enzyme fragments of adenoviral DNA are separated according to size by agarose gel electrophoresis and transferred to nitrocellulose filters by the Southern blotting technique.[3] Unlabeled RNA from cells lytically infected with an Ad2–SV40 hybrid virus is hybridized to filters containing immobilized fragments of adenoviral DNA. Viral mRNAs hybridize to complementary sequences leaving, in the case of the hybrid mRNAs, their SV40 sequences as single-stranded tails. After washing and a second round of hybridization with ^{32}P-labeled SV40 DNA, the filter is established as an autoradiograph. Bands which appear in the final autoradiograph represent fragments containing adenoviral DNA sequences which, in part, serve as template for the synthesis of the hybrid mRNA.

molten agarose is used to effect a seal between the agarose strip containing denatured DNA and the strand-separating gel. Electrophoresis is carried out using phosphate buffer (36 m*M* Tris-HCl, 30 m*M* NaH_2PO_4, 1 m*M* EDTA, pH 7.7)[6] at 1.5 V/cm for the appropriate time after which the separated strands of DNA are visualized by staining with ethidium bromide. After denaturation (see below) and transfer to nitrocellulose, filters containing separated viral DNA strands are baked at 80° for 2 hr. Each preparative gel yields a nitrocellulose filter which is dissected to yield 12–15 individual strips for hybridization.

Blotting. Gels containing DNA fragments or separated viral DNA strands are submersed in 0.2 *M* NaOH, 0.6 *M* NaCl (gel soak I) for 45 min at room temperature. After rinsing in distilled water, gels are transferred to a bath containing 1 *M* Tris-HCl (pH 7.4) and 0.6 *M* NaCl (gel soak II). DNA is transferred onto a sheet of nitrocellulose (B6, Schleicher and Schüell), using essentially the method of Southern.[3] DNA is immobilized by baking nitrocellulose filters at 80° for 2 hr in a vacuum oven.

Isolation of Restriction Enzyme Fragments of Viral DNA. Restriction enzyme fragments of viral DNA separated by agarose gel electrophoresis are visualized by staining with ethidium bromide and examination using ultraviolet illumination. Individual fragments are cut from the gel using a clean scalpel blade. DNA is eluted by the addition of 2 volumes of elution buffer (0.15 *M* NaCl, 10 m*M* Tris-HCl, pH 7.6, 0.001 *M* EDTA). After three strokes in a glass dounce homogenizer the DNA–agarose suspension is extracted for 2 hr at 4° in phenol saturated with 0.5 *M* Tris-HCl, pH 7.6, 0.15 *M* NaCl, 0.001 *M* EDTA. After centrifugation, the supernatant is extracted once more with phenol, once with chloroform, and finally pre-

cipitated by the addition of 2 volumes of ethanol. To ensure purity we routinely pass semipurified fragments through a second agarose gel and isolate fragments as described above. Finally the DNA is precipitated by the addition of 2 volumes of ethanol at −20°.

Nick Translation. Fragments of DNA or intact viral DNA are labeled *in vitro* with [α-^{32}P]deoxyribonucleoside triphosphates by the nick-translation reaction of *E. coli* polymerase I[8] using the conditions established by Rigby *et al.*[9] The radiolabeled DNAs are purified by G-50 Sephadex chromatography. Denaturation is achieved by incubation of labeled DNA in 0.2 *M* NaOH for 10 min at room temperature followed by rapid cooling in ice and neutralization with HCl.

Sandwich Hybridization Reagents

Filter presoak rinse (Denhardt[10])
- Polyvinylpyrrolidone, 0.02% (w/v)
- Ficoll, 0.02% (w/v)
- Bovine serum albumin, 0.02% (w/v)
- 6 × SSC

First stage (RNA) hybridization mix
- Polyvinylpyrrolidone, 0.02%
- Ficoll, 0.02%
- Bovine serum albumin, 0.02%
- SDS, 0.5%
- EDTA, 0.001 *M*
- 6 × SSC
- Unlabeled RNA

Filter washing solution
- 2 × SSC
- SDS, 0.5%

Second stage (^{32}P-labeled DNA) hybridization mix
- Polyvinylpyrrolidone, 0.02%
- Ficoll, 0.02%
- Bovine serum albumin, 0.02%
- SDS, 0.5%
- EDTA, 0.001 *M*
- ^{32}P-Labeled denatured viral DNA 1 μg

Sandwich Hybridization. To avoid problems with RNase contamination all glassware is baked in a hot air sterilizer at 160° for 2 hr and all solutions autoclaved at 15 lb for 20 min.

[8] R. B. Kelly, N. R. Cozzarelli, M. P. Deutscher, I. R. Lehman, and A. Kornberg, *J. Biol. Chem.* **245**, 39–45 (1970).
[9] P. W. J. Rigby, M. Dieckmann, C. Rhoades, and P. Berg, *J. Mol. Biol.* **113**, 237–251 (1977).
[10] D. Denhardt, *Biochem. Biophys. Res. Commun.* **23**, 641–646 (1966).

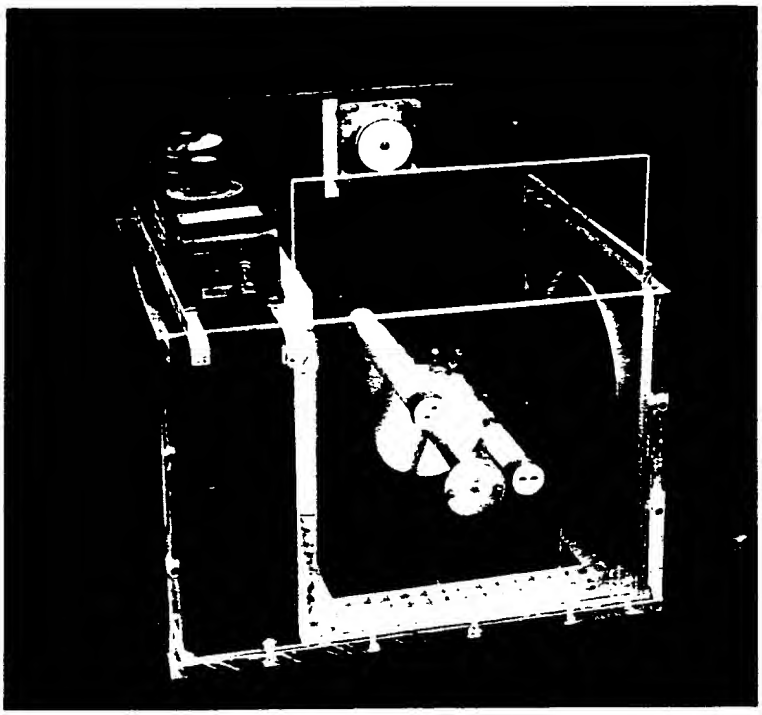

FIG. 3. A convenient hybridization water bath. The hybridization unit, made of Plexiglas (or equivalent), is composed of two compartments. The water-tight outer shell is about 30 cm long, 40 cm wide, and 25 cm high. The inner unit, which can be removed for easy access, houses a wheel with spring clamps to support eight hybridization tubes set in a horizontal position. The wheel is driven at 4 rpm by a suitable gear motor via a connecting chloroprene (neoprene) (O) ring belt. An 800 W laboratory heater-stirrer fitted with a propeller is mounted on the outer shell of the bath and allows accurate (±1°) control of temperature.

Baked nitrocellulose filters containing immobilized DNA are wetted with 6 × SSC, rolled into cylinders and inserted into 150 × 25 mm test tubes. Before hybridization filters are presoaked in "filter presoak rinse" for between 3 and 9 hr at 65°. Pellets of ethanol-precipitated RNA are dried and dissolved in a small volume of 10 m*M* Tris-HCl, pH 7.5, 0.001 *M* EDTA which is added to "first stage hybridization mix" to give a final volume of 3–4 ml. The tubes are sealed with Teflon stoppers and tape and set in a horizontal position on a rotating wheel submersed in a water bath at 65°. The apparatus which was constructed in our laboratory for this purpose is shown in Fig. 3. After hybridization for 12–16 hr, tubes are removed and the hybridization fluid discarded and replaced with "filter wash solution." Filters are washed exhaustively for 4–6 hr at 65° in sev-

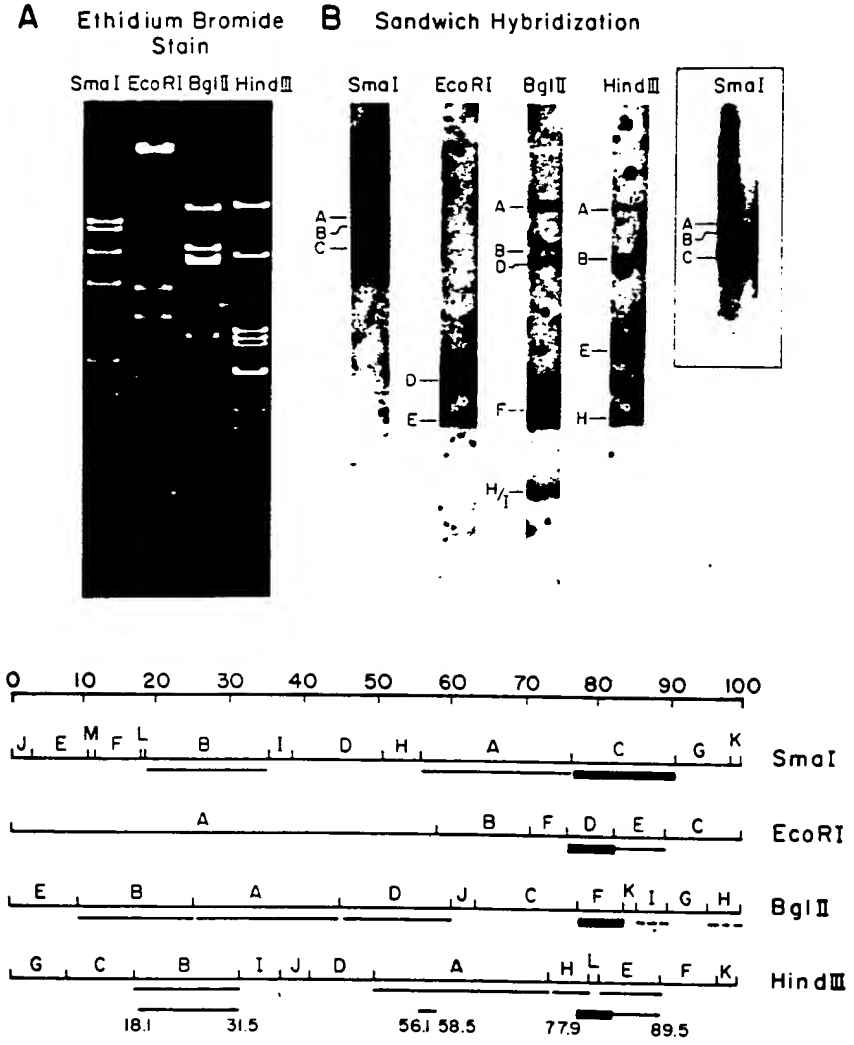

FIG. 4. Analysis of Ad2-SV40 hybrid mRNAs by sandwich hybridization. Late cytoplasmic (20 hr) RNA isolated from CV1 cells infected with Ad2+ND1 (see Fig. 1) is hybridized to restriction enzyme fragments of Ad2 DNA immobilized on a nitrocellulose filter. Sandwich hybridization is carried out using ^{32}P-labeled SV40 DNA as described in the text. (A) represents the original ethidium bromide-stained gel. (B) Autoradiograph of sandwich hybridization. Bands which appear in the final autoradiograph (exposed for 8 days using Kodak No Screen XR-1 film) are identified by reference to the original ethidium bromide-stained gel. Those regions of the viral genome which are complementary to sequences contained within the hybrid mRNA (the main body of the mRNA and the 5′ leader sequences) are indicated on the restriction enzyme maps included at the bottom of this figure. From Dunn and Hassell.[1]

eral changes of filter wash solution. At the end of this period the washing solution is replaced with 3 ml of "second stage hybridization mix" containing up to 1 μg denatured ^{32}P-labeled viral DNA or specific viral DNA fragments labeled *in vitro* by nick translation (specific activity $5 \times 10^7 - 1 \times 10^8$ cpm/μg).

Hybridization is carried out at 65° for 12–16 hr, after which filters are exhaustively washed in filter wash solution for 4–6 hr at 65° . After finally rinsing in 2 × SSC, filters are air dried, mounted on Whatman 3M filter paper, and established as autoradiographs.

A typical analysis of sandwich hybridization using cytoplasmic RNA prepared from cells infected with Ad2$^+$ND1 is shown in Fig. 4.

Sandwich Hybridization to Separated Strands of Viral DNA Fragments. The capacity to separate the strands of certain viral DNA fragments by electrophoresis in agarose gels[6,7] and subsequently to transfer these sequences to a nitrocellulose support allows hybridization to be localized within the "fast" or "slow" migrating viral DNA strands. In the case of well-studied viruses such as adenovirus it is known whether the fast and slow migrating strands of many specific DNA fragments direct the synthesis of either rightward or leftward transcripts on the complete viral genome.

Although the single-stranded DNA within the strand-separating gels is itself available for hybridization after transfer to nitrocellulose, there is always a certain amount of reannealing of denatured strands which routinely run as a slow migrating duplex. Ordinarily these reannealed sequences are unavailable for further hybridization; however, we have found that denaturation of these sequences and subsequent hybridization to them provides a useful reference point on the final autoradiograph for assignment of analytical hybridization to the fast or slow migrating strands of viral DNA (see Fig. 5). To this end we routinely denature the contents of the strand separating gel in gel soak I (see Experimental Procedures, section on blotting) for 45 min at room temperature and then gel soak II for 45 min at room temperature before transferral to a nitrocellulose filter in precisely the manner described for gels containing denatured fragments of viral DNA.

General Considerations

Several factors contribute to the overall sensitivity of the sandwich hybridization technique. Of primary importance is the integrity of the unpaired RNA tails during and after the first stage hybridization. In this respect, maintenance of the nitrocellulose filters in a RNase-free environment throughout the entire period of hybridization is crucial. Clearly the stability of RNA–DNA hybrids formed under our sandwich hybridiza-

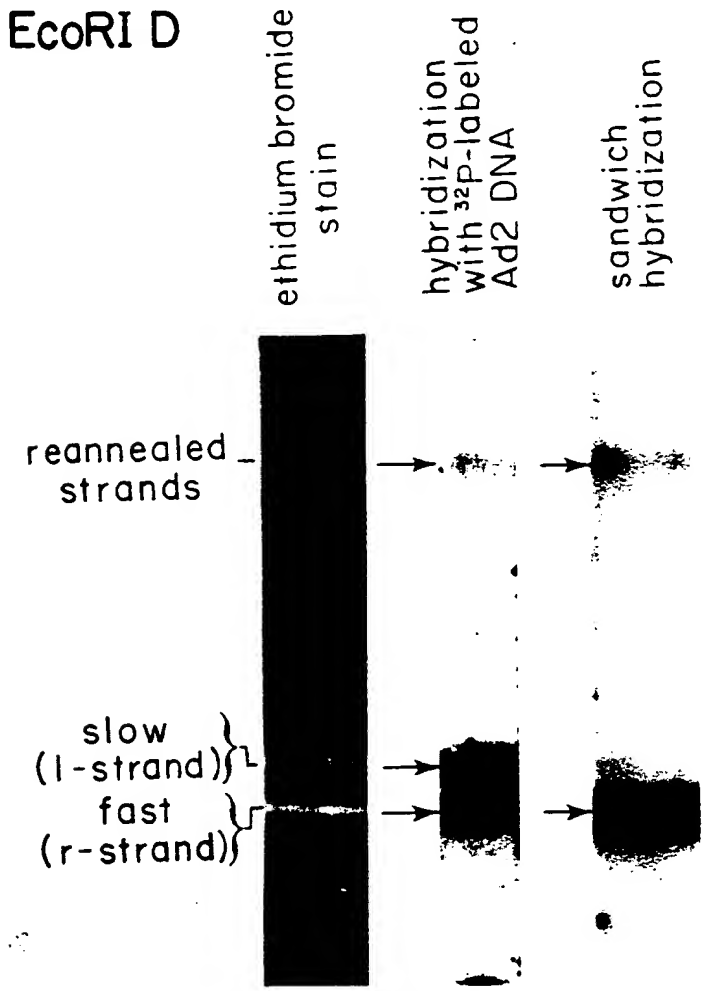

FIG. 5. Sandwich hybridization to separated strands of viral DNA. Late cytoplasmic (20 hr) RNA isolated from CV1 cells infected with Ad2⁺ND1 (see Fig. 1) is hybridized to the separated strands of the *Eco*RI fragment D of Ad2 DNA. Sandwich hybridization is carried out as described in the text using ^{32}P-labeled SV40 DNA and the autoradiograph exposed for 3 days using No Screen film. Hybridization of the hybrid mRNA occurs only to the fast migrating strand of *Eco*RI D previously shown to share sequences with the "r" strand of intact viral DNA.[7] From Dunn and Hassell.[1]

tion conditions is important, and, although we have not investigated this factor, it seems likely that these molecules obey the same set of rules which apply to RNA–DNA hybrids formed in solution or immobilized on nitrocellulose filters. The minimal length of the RNA tail, or the sequences

contained within the tail which are complementary to sequences of the probe, which can form stable RNA–DNA hybrids under the hybridization conditions described here, must be at least 10–15 base pairs (assuming 50% G + C residues).

Because large amounts of viral DNA sequences are contained on the nitrocellulose filters, correspondingly large amounts of RNA can be used during the first stage of hybridization. It is usually feasible, therefore, to achieve the high Cot values for forming RNA–DNA hybrids even when the concentration of specific RNA sequences in the total RNA preparation is low. We have routinely used milligram quantities (1–5 mg/ml) of unfractionated cytoplasmic RNA in our hybridizations which also offers the secondary benefit of protecting the minor concentration of sequences complementary to the probe from degredation with trace amounts of ribonuclease. Where only small quantities of RNA are available, RNase-free carrier RNA (*E. coli* rRNA) can be used as carrier. Ultimately, however, the sensitivity of the technique depends on the concentration of sequences represented in the RNA tails and the specific activity of the ^{32}P-labeled DNA probe used for their detection. In part, high sensitivity can be achieved through the use of DNA fragments labeled to high specific activity with [α-^{32}P]deoxyribonucleoside triphosphates by the nick translation reaction of *E. coli* polymerase.

Future Applications for Sandwich Hybridization

An attractive application of the sandwich hybridization technique takes advantage of the fact that the polyriboadenylic acid residues contained at the 3′ end of mRNAs are not encoded within the structural gene. When mRNAs are hybridized to complementary sequences immobilized on nitrocellulose filters, the poly(A) tract remains unpaired and is available for a second round of hybridization with a probe containing long stretches of ^{32}P-labeled poly(dTTP).

This approach seems particularly valuable for detecting and mapping viral transcripts in virally transformed cells. By using "Southern blots" containing restriction endonuclease-generated fragments of viral DNA, it should be possible within a single sandwich hybridization to determine those areas of the viral genome which are transcriptionally active in any line of transformed cells. Several choices of radioactively labeled probe can be considered useful for the detection of polyriboadenylic acid tracts by sandwich hybridization.

Radioactively Labeled Poly(dT). Homopolymers of radioactively labeled poly(dT) can be synthesized using terminal deoxynucleotidyltransferase and α-^{32}P-labeled thymidine triphosphate. For efficient exten-

sion terminal transferase normally requires exposed 3′ termini which can be provided by a single-stranded homopolymer such as oligo(dT). Alternatively, using the conditions established by Roychoudury *et al.*[11] it is possible to use restriction enzyme fragments of DNA as a primer for terminal transferase without the necessity of exposing the 3′ termini using 5′-exonuclease.

Radioactively Labeled DNA Circles Containing Poly(dT) Tails. When circular DNA molecules, such as SV40, are incubated under certain conditions with terminal deoxynucleotidyltransferase and thymidine triphosphate, the enzyme polymerizes long poly(dT) tails, presumably at single-stranded nicks. Bender and Davidson[12] have successfully utilized these SV40–poly(dT) circles to map the polyadenylated RNAs of several C-type oncornaviruses by direct visualization in the electron microscope. In principle these molecules can be constructed using SV40 DNA radiolabeled with α-^{32}P-labeled deoxynucleoside triphosphates by the nick-translation reaction. Terminal deoxynucleotidyltransferase can then be used to catalyze the addition of several hundred thymidylic acid residues resulting in a molecule of high specific activity ($>10^8$ cpm/μg) for use in the sandwich hybridization experiments described above. Theoretically, circular DNA molecules much larger than SV40 can be used as a template for the addition of poly(dT). This would allow more radioactivity to be associated with the site of a specific DNA–RNA hybrid complex. In this case, the limiting factor will be the stability of the poly(A)–poly(dT) circles and this remains to be determined.

Radioactively Labeled Bacterial Plasmids Containing Tracts of Poly(dT). It should be possible to utilize as a hybridization probe any bacterial plasmid containing an insert of exogenous DNA which was constructed by A–T tailing. The radioactively labeled plasmid (labeled *in vitro* by nick translation using α-^{32}P-labeled deoxynucleoside triphosphates) in its denatured form can be used as a probe where the poly(dT) residues of the plasmid form duplexes with the poly(A) tract at the 3′ end of the mRNA. Preliminary experiments carried out in our laboratory using this approach are encouraging and we are presently investigating the optimal conditions for sandwich hybridization using such a radioactive plasmid as a probe.

Addendum

Recently sandwich hybridization has been used to map a cDNA copy of a specific Ad2–SV40 hybrid mRNA whose 5′ end includes the start of the adenovirus 2 fiber gene.[13] Unlabeled cDNA was synthesized using

[11] R. Roychoudhury, E. Jay, and R. Wu, *Nucleic Acids Res.* **3**, 863–877 (1976).
[12] W. Bender and N. Davidson, *Cell* **7**, 595–607 (1976).
[13] A. R. Dunn, M. B. Mathews, L. T. Chow, J. Sambrook, and W. Keller, *Cell* **15**, 511–526 (1978).

oligo(dT) as a primer in the presence of RNA-dependent RNA polymerase. After hybridization to restriction enzyme fragments of adenovirus 2 DNA, a second round of hybridization was carried out using ^{32}P-labeled SV40 DNA in precisely the manner described using mRNA as the intermediary in the sandwich technique. The authors were able to show that the cDNA hybridized to adenovirus 2 DNA fragments, which included the four leader sequences known to be associated with adenovirus 2 fiber mRNA.[14,15]

By using a cDNA copy of a specific mRNA for sandwich hybridization, it is possible to overcome the problems associated with RNA degradation by trace amounts of ribonuclease.

[14] L. T. Chow, R. E. Gelinas, T. R. Broker, and R. J. Roberts, *Cell* **12**, 1–8 (1977).
[15] L. T. Chow and T. R. Broker, *Cell* **15**, 497–510 (1978).

[55] 5′ Labeling and Poly(dA) Tailing

By P. G. Boseley, T. Moss, and M. L. Birnstiel

Here we describe two methods of ordering DNA restriction fragments. Both are based on terminal labeling and subsequent partial digestion by restriction enzymes. The first method utilizes radioactive terminal labeling and is suitable for the rapid preparation of a restriction map.[1,2] The second method utilizes poly(dA) terminal labeling for the preparation of an ordered set of overlapping fragments. Such ordered fragments are ideal for the base sequence analysis of a DNA molecule using the Maxam and Gilbert procedure.[3] The technique is especially useful for DNA containing repetitive sequences.[4]

Partial Digestion Mapping by Radioactive Terminal Labeling

The principle of the method is the partial digestion of a DNA fragment with a single labeled terminus and its subsequent electrophoresis on agarose gels. The partial digestion results in a complete spectrum of products, but a simple overlapping series, all with a common labeled terminus, can be visualized after gel electrophoresis and autoradiography (see Fig. 1). The relative mobility of each labeled fragment is compared with those of molecular weight standards to determine the restriction sites distance

[1] H. O. Smith and M. L. Birnstiel, *Nucleic Acids Res.* **3**, 2387 (1976).
[2] P. Botchan, R. H. Reeder, and I. B. Dawid, *Cell* **11**, 599 (1977).
[3] A. M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).
[4] T. Moss, P. G. Boseley, and M. L. Birnstiel, in preparation.

ISBN 0-12-181965-5

Methods in Enzymology

Volume 65

Nucleic Acids

Part I

EDITED BY

Lawrence Grossman

DEPARTMENT OF BIOCHEMISTRY
THE JOHNS HOPKINS UNIVERSITY
SCHOOL OF HYGIENE AND PUBLIC HEALTH
BALTIMORE, MARYLAND

Kivie Moldave

DEPARTMENT OF BIOLOGICAL CHEMISTRY
CALIFORNIA COLLEGE OF MEDICINE
UNIVERSITY OF CALIFORNIA
IRVINE, CALIFORNIA

1980

ACADEMIC PRESS

A Subsidiary of Harcourt Brace Jovanovich, Publishers

New York London Toronto Sydney San Francisco

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data
Main entry under title:

Nucleic acids.

(Methods in enzymology, v. 12, 20–21, 29–30, 65)
Pts. C, E–F have title: Nucleic acids and protein synthesis: with editor's names in reverse order on t. p.
Includes bibliographical references.
1. Nucleic acids. 2. Protein biosynthesis. I. Grossman, Lawrence, Date ed. II. Moldave, Kivie, Date ed. III. Title: Nucleic acids and protein synthesis. IV. Series: Methods in enzymology. v. 12 [etc.] [DNLM: 1. Nucleic acids––Biosynthesis. 2. Proteins––Biosynthesis. W 1 Me9615K v. 30 1974 QU 55 N964 1974]
QP601.M49 vol. 12, etc. 574.1'925'08s [QP620]
ISBN 0–12–181965–5 (v. 65) [574.8'732] 74–26909

PRINTED IN THE UNITED STATES OF AMERICA

80 81 82 83 9 8 7 6 5 4 3 2 1

Table of Contents

Section IV. Purification and Properties of Enzymes Acting at Sites with Altered Bases

Section V. Endonuclease Cleavage Mapping Techniques

Section VIII. Nucleotide Sequencing Techniques

Section IX. Localization of Functional Sites on Chromosomes

Contributors to Volume 65

Article numbers are in parentheses following the names of contributors.
Affiliations listed are current.

KAN L. AGARWAL (19), *Department of Biochemistry, University of Chicago, Chicago, Illinois 60637*

CHANDER P. BAHL (78), *Cetus Corporation, Berkeley, California 94710*

SUSAN M. BERGET (69), *Department of Biochemistry, Rice University, Houston, Texas 77001*

ARNOLD J. BERK (69), *Department of Microbiology, University of California, Los Angeles, California 90024*

KATHLEEN L. BERKNER (5), *Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

THOMAS A. BICKLE (11, 16), *Department of Microbiology, Biozentrum, University of Basel, CH-4056 Basel, Switzerland*

M. L. BIRNSTIEL (55), *Institut für Molekularbiologie II der Universität Zürich, Hönggerberg, 8093 Zürich, Switzerland*

ROBERT BLAKESLEY (23), *Bethesda Research Laboratory, Rockville, Maryland 20850*

P. G. BOSELEY (55), *Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom*

SIERD BRON (15), *Department of Genetics, Centre of Biological Sciences, University of Groningen, 9751 NN Haren (Gn), Kerklaan 30, The Netherlands*

R. BROUSSEAU (61), *Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A OR6, Canada*

NIGEL L. BROWN (48), *Department of Biochemistry, University of Bristol, Bristol Medical School, Bristol BS8 1TD, England*

A. G. BRUCE (9), *Department of Biochemistry, University of Illinois, Urbana, Illinois 61801*

A. I. BUKHARI (52), *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724*

JOHN CAMERON (49), *Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305*

GORDON G. CARMICHAEL (47), *Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115*

JAMES F. CATTERALL (21), *Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030*

GEORGE CHACONAS (10), *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724*

MARK D. CHALLBERG (6), *Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

THOMAS R. CHAUNCEY (19), *Department of Biochemistry, University of Chicago, Chicago, Illinois 60637*

JACK G. CHIRIKJIAN (23), *Department of Biochemistry, Georgetown University Medical Center, Washington, D.C. 20007*

ALAN D. D'ANDREA (31), *Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115*

KATHLEEN J. DANNA (53), *Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309*

SANTANU DASGUPTA (51), *Biophysics Laboratory and Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706*

RONALD W. DAVIS (49), *Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305*

BRUCE DEMPLE (29), *Department of Biochemistry, University of California, Berkeley, California 94720*

ASHLEY R. DUNN (54), *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724*